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Effects of LH-RH on Isolated Pituitary Gonadotropic Cells (39165)

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Several investigators have studied FSH and LH release from intact anterior pituitary glands and from dissociated pituitary cells (1–5). The decapeptide luteinizing hormone-follicle stimulating hormone releasing factor (LH-RH) has been shown to release both LH and FSH (2, 3, 5). The separation of isolated rat anterior pituitary gland cells into enriched fractions has also been reported (6–8). Lloyd and McShan (8) reported that after separation of monodispersed rat pituitary cells by velocity sedimentation at unit gravity, most of the FSH and LH activities were located in the first 260 ml of the gradient, and that the FSH and LH cells were also found in these fractions as shown by electron microscopic examination. This report describes the effects of LH-RH and N^6, 2′-*O*-dibutyryl cyclic adenosine 3′:5′-monophosphate (cAMP) on concentrated gonadotropic cells prepared by velocity sedimentation at unit gravity.

Materials and methods. Pituitary glands from male rats weighing 300–350 g were used (Holtzman Company, Madison, Wisconsin) to prepare monodispersed cells by the method of Lloyd and McShan (8). The cell suspension was placed in a velocity sedimentation chamber and allowed to sediment for 4 hr (8). Briefly, the procedure consisted of introducing the cell suspension in 0.25% bovine serum albumin (BSA) into the chamber under a 15 ml overlay of Eagle's Minimum Essential Medium (EMEM, Grand Island Biological Company). A gradient consisting of 0.5 to 2.0% BSA in EMEM, pH 7.4, was generated with a peristaltic pump. After allowing the cells to sediment for 4 hr, 10-ml fractions were collected and the cells were concentrated by centrifugation at 200 *g*. The number of cells per fraction was counted in duplicate with the aid of a hemocytometer.

Releasing factor studies. After velocity sedimentation, between 5×10^4 and 1×10^5 of the enriched gonadotropic cells were placed on sterile cover slips in 35 × 10 mm plastic dishes (Falcon) at 37° over a humidified atmosphere of air/CO_2 (95/5). The incubation medium consisted of Medium 199 (100 ml) containing lyophilized horse and fetal calf sera, 5 and 10 g, respectively; penicillin (162 U/ml) and streptomycin (0.1 mg/ml) at pH 7.4. The cells were allowed to equilibrate for 2 hr before addition of synthetic LH-RH. Synthetic LH-RH, Code 3252-146, was a gift from Dr. W. F. White of Abbott Laboratories. In one series of experiments LH-RH was added at a concentration of 100 ng/ml, while saline was added to control dishes. After 5 and 30 min of incubation, the cells were quickly centrifuged for 5 min at 200 *g*, and the medium and cells were separated and assayed for LH and FSH activities by their respective radioimmunoassays (RIA). In another series of experiments, the LH-RH was added to the medium at concentrations of 1, 10, and 100 ng/ml along with 10^{-3} *M* cAMP (Sigma), and the cells were maintained in culture for 24 hr. At the end of this period, the cell suspension was centrifuged and both cells and medium were assayed for LH activity.

Radioimmunoassay. Radioimmunoassay materials for rat FSH and LH were obtained from NIAMD, NIH. The double antibody method of Daane and Parlow for FSH was used (9). A 1:4000 dilution of NIAMD-anti-rat FSH Serum S-6 at two dose levels in duplicate was used for each sample. Serial dilutions of rat pituitary homoge-

nates were parallel to the standard curve from 20 to 100 ng of FSH. Cross-reaction with NIAMD rat LH for radioiodination was 2.5% on a weight basis and, with NIAMD rat STH for radioiodination, was 0.11% on a weight basis. The coefficient of variation using two separate pituitary homogenates as an internal standard was 4.0 and 7.5% for four assays. Results were expressed in terms of NIAMD-rat FSH-RP-1. Radioimmunoassay for rat LH using the NIAMD kit has already been reported (8).

Results. Examination of the cells by light microscopy after velocity sedimentation showed the same size distribution as well as FSH and LH activities in the various fractions as previously reported (8). FSH and LH cells which had 57% of the total gonadotropic activity as determined by RIA were found primarily in the first 260 ml of the bovine serum albumin gradient after velocity sedimentation for 4 hr. These enriched gonadotropic cells were used to study hormone release after addition of LH-RH or cAMP.

After 5 min of incubation with LH-RH, a significant increase in the secretion of LH or FSH was not observed. However, after 30 min there was a significant increase in both LH and FSH in the medium. There were no significant differences in the LH and FSH values in the cells after 5 and 30 min of incubation, although the LH and FSH contents in cells were lower in the experimental as compared to the control groups (Table I).

After 24 hr of incubation, the dishes with 1 and 10 ng/ml of LH-RH had a slight, but nonsignificant increase in the amounts of LH found in the medium. However, 100 ng/ml of LH-RH and 10^{-3} *M* cAMP both caused significant increases in the medium LH over control values. After 24 hr of incubation in the presence of LH-RH or cAMP, the amount of LH found in the cells was not significant among the different groups (Table II).

Discussion. In this study, there was no significant response after addition of LH-RH for 5 min in the concentrated gonadotropic cells *in vitro*. The large amounts of LH and FSH detected in the control medium most likely reflect hormones released when the cells were preincubated at 37° after being at 4° for 4 hr. After 24 hr of incubation with three different concentrations of LH-RH, the cells with 1 and 10 ng/ml had approximately the same amounts of LH in the medium. However 100 ng/ml of synthetic LH-RH caused a significantly greater release of both FSH and LH. The poor response of dissociated cells to secre-

TABLE II. EFFECTS OF LH-RH AND cAMP ON THE RELEASE OF LH FROM CONCENTRATED GONADOTROPIC CELLS *IN VITRO*.[a]

Treatment	μg LH/dish in medium	μg LH/dish in cells
Control	10.12 ± 0.35	2.45 ± 0.48
1 ng/ml LH-RH	11.01 ± 0.46	2.32 ± 0.48
10 ng/ml LH-RH	11.08 ± 0.95	1.78 ± 0.11
100 ng/ml LH-RH	21.60 ± 2.47**	1.95 ± 0.05
10^{-3} *M* cAMP	14.75 ± 0.75*	2.22 ± 0.41

[a] Statistical analysis was done by the one-sided multiple comparison test of Dunnett. (* $P < 0.05$; ** $P < 0.01$). Values are the mean ± SEM for four experiments. Aliquots of 5×10^4 to 1×10^5 cells/dish were used.

TABLE I. EFFECT OF LH-RH ON THE RELEASE OF LH AND FSH FROM CONCENTRATED GONADOTROPIC CELLS *IN VITRO*.[a]

Treatment	Incubation time	μg LH/dish in medium	μg LH/dish in cells	μg FSH/dish in medium	μg FSH/dish in cells
Control 100 ng/ml LH-RH	5 min (3)	6.67 ± 0.83	3.45 ± 1.11	2.03 ± 0.29	2.30 ± 0.16
		6.71 ± 0.62	3.30 ± 0.29	2.19 ± 0.38	1.87 ± 0.24
Control 100 ng/ml LH-RH	30 min (3)	6.33 ± 0.34	3.07 ± 0.74	4.01 ± 0.25	2.13 ± 0.15
		9.07 ± 0.71*	2.01 ± 0.22	4.73 ± 0.36*	2.40 ± 0.32

[a] Statistical analysis was done by Student's *t* test (*$P < 0.05$). Numbers in parentheses indicate number of experiments. Aliquots of 5×10^4 to 1×10^5 cells/dish were used.

tagogues immediately after enzyme treatment and their improved response after incubation for a few days in culture has been observed (4, 12). This probably results from damage to the cell surface during dissociation (12). Since 100 ng/ml of LH-RH and 10^{-3} *M* cAMP did produce a significant increase of LH and FSH in the medium, it can be concluded that the cells show a partial response to LH-RH and cAMP after enzyme dissociation followed by velocity sedimentation for 4 hr. Shiino *et al.* (10) and Mendoza *et al.* (11) observed responses of the pituitary to LH-RH, 2.5, 3, and 15 min after injection of this hormone *in vivo*. However, with the isolated cells, hormone release was observed after 30 min of incubation with LH-RH.

The total amounts of hormone present in the medium after 24 hr of incubation with 100 ng/ml indicate that this concentration of LH-RH caused a highly significant ($P < 0.01$) increase in LH release. This observation agrees with previously reported data using more chronic treatment regimens with LH-RH (4). This could indicate a stimulation of synthesis of new hormone and a redistribution of these hormones from an intracellular to an extracellular pool, which in this case would be the culture medium.

Incubation with cAMP resulted in an increase of LH in the medium after 24 hr of incubation. Borgeat *et al.* (2) have reported the specific stimulation of adenylate cyclase activity in gonadotropic cells. Since this stimulation of the adenylate cyclase system by LH-RH is specific for the gonadotropic cells (2), it is thought that this is the mechanism by which LH-RH brings about granule release. The observation that cAMP resulted in an increased secretion of LH in these experiments would support the above concept.

Summary. Rat anterior pituitary glands were dissociated with Pronase and the cells were separated by velocity sedimentation at unit gravity. After 30 min of incubation of the enriched gonadotropic cells with LH-RH, there was a significant increase in LH and FSH in the incubation medium. LH-RH (100 ng/ml) and 10^{-3} *M* cAMP both caused significant increases in LH in the incubation medium after 24 hr of incubation.

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